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Review

A structural perspective on MHC class I recognition by killer cell immunoglobulin-like receptors

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Abstract

Killer cell immunoglobulin-like receptors (KIR) play a critical role in the regulation of natural killer (NK) cell activity through their recognition of class I MHC molecules expressed on target cells. KIR recognition provides vital information to NK cells about whether a target cell should be lysed or spared. Understanding the molecular mechanism of this recognition has remained a strong focus of investigation. This has resulted in the crystal structures of several members of the KIR family and more recently the determinations of the three dimensional structures of KIR2DL2 and KIR2DL1 complexed with their respective ligands, HLA-Cw3 and HLA-Cw4. A strong structural conservation has been revealed both in the receptor design and in the overall mode of KIR binding to class I molecules. Nevertheless, distinct differences in the receptor binding sites allow for high specificity between ligands. Furthermore, unexpected similarities with T-cell receptor (TCR) recognition of MHC molecules are also observed. The detailed interactions between KIR and HLA-C molecules and their functional implications will be reviewed here. Published by Elsevier Science Ltd.

Keywords: NK cell receptors; Receptor recognition; Allotype specificity; KIR; HLA-C

1. Introduction

Natural killer (NK) cells constitute a key component of the innate immune system and as such are able to respond more rapidly than T- or B-cell-mediated adaptive immunity. The cytolytic activity and cytokine production of NK cells is tightly regulated by an array of activating and inhibitory receptors on the cell surface (Raulet et al., 2001). A rather diverse collection of activating receptors engage various surface antigens on target cells, as well as certain class I MHC molecules. To date the ligands for many of these receptors remain unknown. In contrast, inhibitory receptors primarily recognize class I MHC molecules (Lanier, 1998; Long, 1999). Structurally, they comprise two distinct superfamilies, C-type lectin-like (Ly49 and CD94/NKG2) and immunoglobulin (Ig)-like (killer cell Ig-like receptors (KIR) and Ig-like transcripts (ILT) or leukocyte Ig-like receptors (LIR)). Ly49 and KIR both recognize classical class I MHC molecules and are believed to be functional orthologs. KIR exists exclusively in human and other primates, whereas Ly49 is found only in mice (Karlhofer et al., 1992). The heterodimeric CD94/NKG2 receptors, however, are conserved throughout the species along with their ligands, the non-classical class I MHC molecules, HLA-E in humans and Qa-1 in mice (Borrego et al., 1998; Braud et al., 1998; Lee et al., 1998; Vance et al., 1998).

2. The KIR superfamily

KIR are type I transmembrane glycoproteins with two to three extracellular C2-type Ig domains (Wagtmann et al., 1995a; Colonna and Samaridis, 1995; D'Andrea et al., 1995) and include both inhibitory and activating forms (Moretta et al., 1993, 1995; Litwin et al., 1994; Dohring et al., 1996a). Activating forms have a relatively short cytoplasmic tail and contain a positively charged residue in the

Abbreviations: NK, natural killer; KIR, killer cell immunoglobulin like receptor; Ig, immunoglobulin; TCR, T-cell receptor; CTLR, C-type lectin-like receptor; ILT, Ig-like transcript; LIR, leukocyte Ig-like receptor; LAIR, leukocyte-associated Ig-like receptor; PIR, paired Ig-like receptor; rms, root mean square; ITIM, immunoreceptor-tyrosine based inhibitory motif; LRC, leukocyte receptor complex; FcR, Fc receptor; GAV, GAVDPLLAL peptide; QYD, QYDDAVYKL peptide; SPR, surface plasmon resonance

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transmembrane region that interacts with specific activating adaptor proteins. The inhibitory receptors have a longer cytoplasmic tail containing immunoreceptor-tyrosine-based inhibitory motifs (ITIM) (Lanier, 1998; Long, 1999). A nomenclature was introduced to name the members of KIR according to the number of Ig domains present in each gene and whether they possess long (ITIM containing) or short cytosolic tails (Valiante et al., 1997). For example, KIR3DL1 represents an inhibitory receptor with three Ig domains and a 'long' intracellular tail possessing ITIM sequences, whereas KIR3DS1 represents a non-inhibitory form with a 'short' cytoplasmic tail containing no ITIM segments. The consecutive domains are named D1 and D2 in KIR2D and D0, D1 and D2 in KIR3D receptors, respectively. Members of KIR family share greater than 90% sequence identity. With the exception of KIR2DL4 and KIR2DL5, the second and third domains of the three domain KIRs are best aligned against the first and second domains of the two domain KIRs. KIR2DL4 and KIR2DL5 each possess D0 and D2 domains. Human KIR are encoded by more than a dozen genes located in the leukocyte receptor complex (LRC) region on chromosome 19q (Steffens et al., 1998; Trowsdale et al., 2001; Wilson et al., 1997). A similar number of KIR genes are observed in other primate species (Khakoo et al., 2000). Recently, a leukocyte antigen CD based nomenclature was recommended to replace the KIR, ILT and LIR nomenclature (Andre et al., 2001). Following this nomenclature, KIRs are known as CD158 and alphabetized according to the centromeric–telomeric position of their genes on chromosome 19 (e.g. CD158a, CD158b, etc.).

Several other KIR-like cell surface receptors, have also been discovered including ILT (or LIR) that contain two or four Ig-like domains (Samaridis and Colonna, 1997; Borges et al., 1997; Colonna et al., 1997), leukocyte-associated Ig-like receptors (LAIR-1 and -2) that contain a single Ig-like domain (Meyaard et al., 1997) and paired Ig-like receptors (PIR-A and -B) that contain six Ig-like domains (Arm et al., 1991; Kubagawa et al., 1997; Hayami et al., 1997). These receptors display 35–50% sequence identity with KIR and are part of KIR superfamily. A more distantly related set of proteins are the Ig-like Fc receptors (FcαR, FcγR-I, -IIa, -IIb, -III and FcεR-I), which display less than 20% sequence identity with KIR but nonetheless share a structural fold similar to KIR.

2.1. The three dimensional structure of KIR

To date, the crystal structures of the extracellular domains of three members of KIR family, KIR2DL1, KIR2DL2 and KIR2DL3, have been published (Fan et al., 1997; Snyder et al., 1999; Maenaka et al., 1999b). Overall, the KIR fold is very similar to the C2-type Ig-like fold observed in the hematopoietic receptors. However, three differences are noteworthy. The first difference involves the pairing of two β-strands. In hematopoietic receptors, β-strand A pairs with β-strand B, whereas in KIR structures the first strand splits into two β-strands, A and A′, which hydrogen bond with the B and G strands, respectively, resulting in a "strand switching". This strand switching is likely attributable to the presence of a *cis*-proline residue in the first strand that is conserved in all members of KIR. Secondly, the KIR fold contains an additional short D strand that is absent in other C2-type Ig-like folds. Lastly, in addition to the slight differences in strand arrangement, the KIR structures possess unique tertiary packing. In particular, the hinge angle between the N-terminal D1 and C-terminal D2 domains is acute. Not only is this angle smaller than those observed in hematopoietic receptors such as the human growth hormone receptor, but it is also smaller than the hinge angles of other two-domain Ig-like structures, such as the V and C domains of T-cell antigen receptors and the V and C_{H1} domains of antibodies. The hinge angle of KIR varies from 66° in KIR2DL1 to 81° in KIR2DL2 and KIR2DL3. It is stabilized by a highly conserved interdomain hydrophobic core (hinge core) that consists of Leu17, Met69, Val100, Ile101, Thr102, His138, Phe178, Ser180, Pro185, Tyr186 and Trp188. An interdomain salt bridge between Asp98 and Arg149, conserved in all KIR family sequences, also appears to restrict the hinge angle. The D1 and D2 domains themselves share 40% sequence identity and appear to be the result of gene duplication. The superposition between the Cα atoms of D1 and D2 domains results in a root mean square (rms) deviation of approximately 1.2 Å. Apart from the differences in the hinge angle, the three KIR structures are nearly identical with rms deviations less than 1 Å between the Cα atoms of their respective domains.

2.2. Structures of other KIR-like immune receptors

The structure of the ligand binding domains (D1 and D2) of ILT-2 (LIR-1) has recently been determined (Chapman et al., 2000). ILT-2 is an inhibitory receptor expressed on monocytes, B-cells, dendritic cells and subsets of NK- and T-cells (Samaridis and Colonna, 1997). The sequence identities between KIR and ILT genes are about 40% and both domains 1 and 2 of LIR-1 possess the KIR type Ig fold including strand switching in the first β-strands. Distinct structural differences are observed, however, between LIR-1 and KIRs (Chapman et al., 2000). In particular, LIR-1 has two unique short 3_{10} helices in each domain. One replaces the C′ strand in the D1 domain and the C-terminal end of C′ strand in the D2 domain found in KIR and the other is situated between the E and F strands in the D1 domain and between the F and G strands of the D2 domain. A short left-handed type II polyproline-like helix is also found in the FG loop of the D1 and D2 domains. The interdomain region of LIR-1 consists of predominantly hydrophobic residues, forming a hinge core similar to that of KIR that stabilizes the relative D1–D2 orientations. However, a conserved interdomain salt bridge in KIR between Asp98 and Arg149 is absent in the LIR-1 structure. This may contribute to the slightly larger hinge angle of LIR-1 (88°).

More distantly related to KIR and ILT are the Ig-like Fc receptors. The fold of Fc receptors, (FcR) as seen in the structures of FcγR-IIa, FcγR-IIb, FcγR-III and FcεR-I, resembles that of KIR despite sharing less than 20% sequence identity with KIR (Maxwell et al., 1999; Sondermann et al., 1999; Garman et al., 1998; Zhang et al., 2000). For example, the characteristic strand switch found in the first β-strand of KIR domains is also present in the FcR structures. Furthermore, the interdomain region of FcR also contains a hydrophobic hinge core and an interdomain salt bridge, although the residues that contribute to the hinge core and the salt bridge are not conserved between FcR and KIR. The individual domains of FcR can be superimposed to those of KIR resulting in rms differences between Cα positions of approximately 2.0 Å. In contrast to KIR and LIR-1, the hinge angles in the FcR structures are appreciably smaller, varying between 48 and 55°. Additionally, the D1 domain of FcRs is located on the opposite side of the D2 domain relative to what is observed in KIR molecules.

2.3. HLA ligands of KIR

The identification of an inverse relationship between the target cells lysis susceptibility and the expression level of surface class I MHC molecules on target cells provided some of the first evidence implicating class I molecules as potential ligands for NK cell receptors (Harel-Bellan et al., 1986; Storkus et al., 1987). This conclusion was further strengthened by the observation that transfection of class I genes into a class I deficient target cell was sufficient to protect these cells from NK cell-mediated lysis (Shimizu and DeMars, 1989). Evidence for the involvement of multiple receptors that recognized distinct HLA class I molecules came from investigations of the specificity of cloned NK cells against different allogeneic target cells. Two KIR2DL receptors, originally referred to as p58, were identified and their expression correlated with the allospecificity of cloned NK cells for target cells. In particular, NK clones that expressed the p58 molecule recognized by the EB6 mAb (Moretta et al., 1990, 1993) were unable to lyse target cells expressing the HLA-Cw2, 4, 6, or 15 allotypes. Similarly NK cells expressing the other p58 molecule recognized by the GL183 mAb were unable to lyse target cells expressing HLA-Cw1, 3, 7, or 8 molecules (Winter et al., 1998; Ciccone et al., 1992). Sequence comparison among these allotypic HLA molecules suggested that a dimorphism at residues 77 and 80 in class I MHC heavy chain accounts for the observed NK cell lytic specificities (Colonna et al., 1993a, b). HLA-B molecules with the Bw4 serological epitope were found to contain a similar region shown to confer protection from NK cells bearing the KIR3D receptor (p70) (Litwin et al., 1994; Gumperz et al., 1995). However, HLA-A2403 and -A2501 alleles that also possess the Bw4 epitope are unable to protect target cells from lysis by p70 expressing NK cells, suggesting that other regions of the class I heavy chain are also required for recognition (Gumperz et al., 1995). The p58 receptors have been subsequently renamed as KIR2DL1 (specific for HLA-Cw2, 4, 6, and 15) and KIR2DL2 and KIR2DL3 (specific for HLA-Cw1, 3, 7, and 8). The p70 receptor that interacts with HLA-B alleles of the Bw4 serotype was renamed KIR3DL1 (Wagtmann et al., 1995a,b; Long et al., 1996; Colonna and Samaridis, 1995). The interactions between KIR and their class I HLA-B and -C ligands have been observed directly using soluble forms of receptors (Wagtmann et al., 1995b; Boyington et al., 2000; Fan et al., 1996; Rajagopalan and Long, 1997; Vales-Gomez et al., 1998a; Rojo et al., 1997; Maenaka et al., 1999a). Although, there is no direct binding evidence, some KIR3D receptors have also been suggested to recognize certain HLA-A alleles (Pende et al., 1996; Dohring et al., 1996b). Recently, the ligand for KIR2DL4 has been identified as the non-classical class I molecule HLA-G (Rajagopalan and Long, 1999).

3. Structure of KIR/HLA complexes

The first crystal structure of a KIR/HLA complex determined was that of KIR2DL2 bound with HLA-Cw3 and a nonamer self peptide GAVDPLLAL (GAV) from importin-α1 (Boyington et al., 2000). This structure revealed a common 1:1 binding mode, an elegant system for allotype recognition and limited peptide restriction. The 1:1 stiochiometry is also supported by analytical ultracentrifugation measurements (Sun and Schuck, unpublished data), gel-shift assays and surface plasmon resonance (SPR) studies (Fan et al., 1996; Vales-Gomez et al., 1998b; Maenaka et al., 1999a). KIR is bound across both the α1 and α2 helices of HLA and the C-terminal end of the bound peptide with its long axis nearly orthogonal to the peptide binding cleft (Fig. 1A). This orientation is analogous to TCR/HLA interaction, with the tandem D1 and D2 domains of KIR assuming positions similar to the Vα and Vβ domains of TCRs (Ding et al., 1998) (Fig. 1B). Both KIR and TCRs bury similar amounts of solvent accessible surface area (1500–1800 $Å^2$) and exhibit comparable shape complementarity ($S_c = 0.45$–0.70) when complexed with HLA molecules (Boyington et al., 2000; Garcia et al., 1999). There are, however, unique features in the KIR/HLA recognition. While TCRs interact with peptides at the central P4–P6 positions (Garcia et al., 1996), KIR binding is centered over the P7–P8 positions near the C-terminus of the peptide. The footprint of KIR on HLA is distinct from that of TCRs but overlaps, precluding simultaneous binding on the same HLA molecule. More recently, the related structure of KIR2DL1/HLA-Cw4 with a bound peptide QYDDAVYKL (QYD) was also determined (Fan et al., 2001), verifying the recognition principles observed in the KIR2DL2/HLA-Cw3 structure.

3.1. The KIR/HLA interface

The ligand binding area of KIR comprises six surface loops near the interdomain hinge region. Topologically,

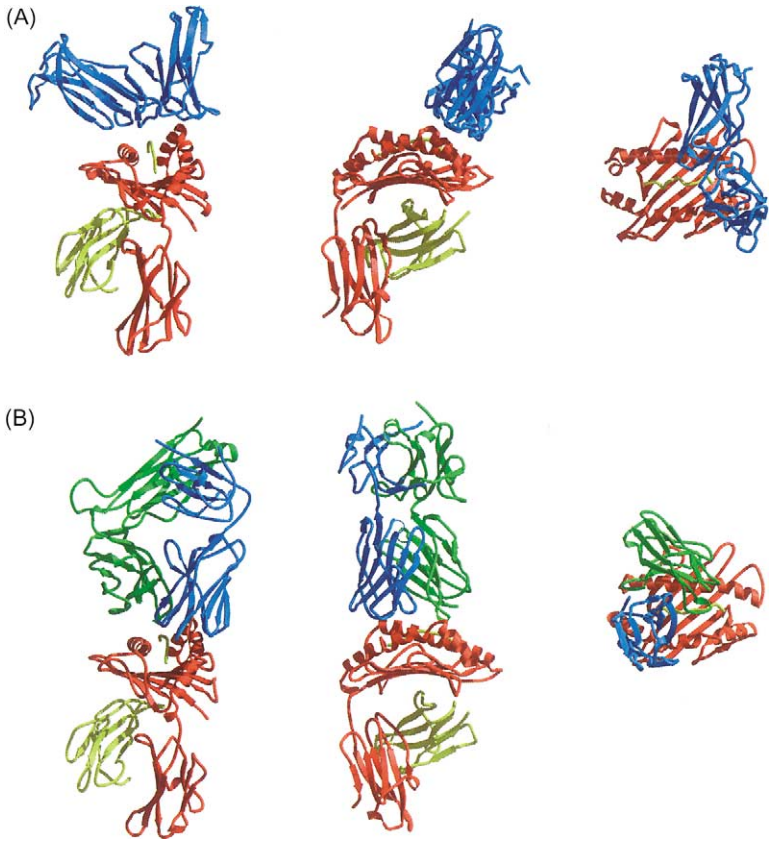

Fig. 1. Comparison of KIR/HLA (A) and TCR/HLA complexes (B). Panels A and B show front, side and top views of the KIR2DL2/HLA-Cw3/GAV (PDB code 1EFX) and B7 TCR/HLA-A2/TAX (PDB code 1BD2) complexes, respectively. The class I MHC molecules are red and yellow ribbons with yellow peptides. KIR2DL2 is blue and the B7 TCR is blue and green. For clarity, the top views show only the α1α2 MHC domains and only the Vα and Vβ domains of the TCR. All molecular figures in this review are created with MOLSCRIPT (Kraulis, 1991) and RASTER3D (Merrit and Murphy, 1994).

these loops are identical to the six ligand binding loops used by many hematopoietic cytokine receptors such as human growth hormone receptor. Three of these loops (A′B, CC′ and EF loops of the D1 domain) interact with the α1 helix of HLA. The other three, the BC, FG loops of the D2 domain and the hinge loop contact the α2 helix of HLA. In contrast to TCR/HLA interfaces that are characterized by predominately non-polar, Van der Waals and hydrogen bonding interactions, the KIR/HLA interface displays striking charge complementarity (Fig. 2). KIR provides six acidic residues and HLA contributes six basic residues to the interface. This results in the formation of four salt bridges between KIR2DL2 and HLA-Cw3 (Glu21–Arg69, Glu106–Arg151, Asp135–Arg145 and Asp183–Lys146) and three salt bridges between KIR2DL1 and HLA-Cw4 (Asp135–Arg145, Asp183–Lys146 and Glu187–Lys80). Additionally, several hydrogen bonds are present at the interface (seven in KIR2DL2/HLA-Cw3 and six in KIR2DL1/HLA-Cw4). Similar charge–charge interactions are observed within the interfaces of the Ly49/H-2D^{d} NK cell receptor complex (Tormo et al., 1999) and the CD2/CD58 adhesion complex (Wang et al., 1999). The significance of charge complementarity at the KIR/HLA interface was investigated by individually mutating three residues of KIR2DL2 (E106A, D135H and D183A) that form salt bridges at the interface (Boyington et al., 2000). Equilibrium binding measurements using SPR techniques revealed drastic reductions in HLA-Cw3 affinity for all three mutants (Table 1), highlighting the importance of these salt bridges and suggesting a high binding energy threshold for recognition. The KIR/HLA interfaces also include two hydrophobic patches that account for about one-third of the interface interactions. These include the aliphatic portions of residue 44, Phe45 and Asp72 from the D1 domain of KIR and the aliphatic portions of HLA residues Arg75, Val76 and Arg79 from the α1 helix. Likewise, the non-polar regions of KIR D2 domain residues Tyr105, Ser132, Phe181 and Asp183 interact with the non-polar regions of Lys146 and Ala149 from the HLA α2 helix. In the KIR2DL2/HLA-Cw3/GAV complex, the second hydrophobic region also includes peptide residues Leu7 and Ala8 and KIR residues Gln71 and Leu104. Substitution of Phe45 with Tyr in KIR, as occurs naturally in KIR2DS2, reduces the HLA affinity of KIR significantly (Winter et al., 1998). This reduced affinity can be attributed to a close packing between Phe45 of KIR and residues Arg75, Val76 and Arg79 of the HLA α1 helix,

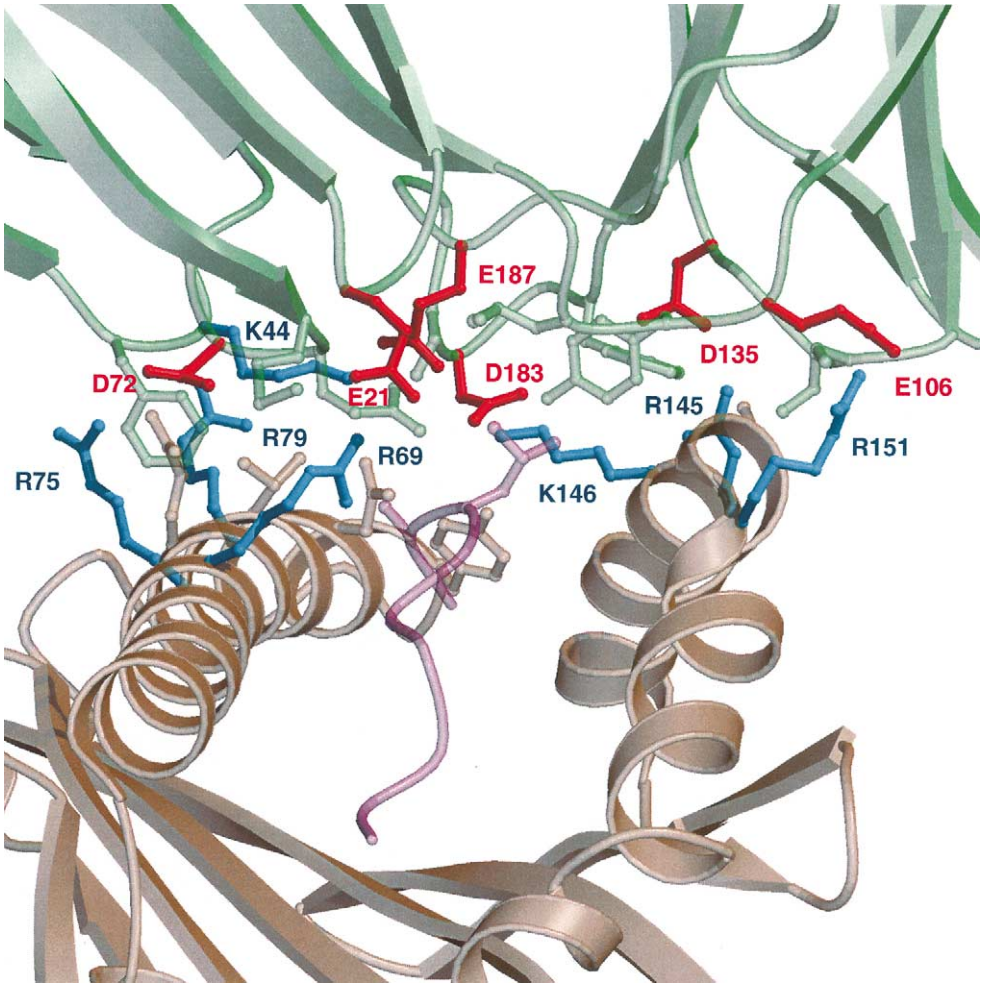

Fig. 2. Charge complementarity between KIR2DL2 and HLA-Cw3. Basic residues are blue, acidic residues are red. KIR and HLA-C are represented by green and brown ribbon models, respectively.

Table 1
Peptide and receptor mutation effects in KIR2DL2/HLA-Cw3 association

Peptide	K_D (μM)	w6/32 binding (%)
Effects of peptide variation in KIR/HLA binding		
GAVDPLLAL (GAV)	9.5	100
GAVDPLLSL (GAV_S)	42.3	147
GAVDPLLVL (GAV_V)	525	130
GAVDPLLYL (GAV_Y)	> 600	130
GAVDPLLKL (GAV_K)	> 600	139
AAADAAAAL (AAA)	48.5	149
TAMDVVYAL (TAM)	38	138
QAISPRTL (QAI)	74	38
HLA-E	> 600	84
KIR Mutant	**K_D (μM)**	
Effects of amino acid substitutions in KIR/HLA association		
Wild type	28	
R33A	30	
K44M	> 400	
Y105A	> 400	
E106A	185	
D135H	> 400	
D183A	> 400	

leaving little space and no suitable hydrogen bonding partner to accommodate the hydroxyl group of Tyr. A similar argument can be made to the KIR2DL1/HLA-Cw4 structure. It is possible that this type of subtle difference in the interface packing accounts for the observed lower affinity in the non-inhibitory versus the inhibitory KIR.

3.2. KIR2DL2/HLA-Cw3 and KIR2DL1/HLA-Cw4 share a common binding mode

Among the common interface residues, 15 of the 18 KIR and 11 of the 12 HLA residues are conserved between the two KIR/HLA complexes. This results in fundamentally identical binding modes (Fig. 3A) guided strongly by charge compensation, specific hydrogen bonds and a unique pattern of non-polar residues. Despite this impressive interface conservation, least squares superposition of the two complexes through their HLA-C α1α2 domains reveals a rotation of KIR2DL1 relative to KIR2DL2 of about 9°. This rotation is centered about the D2 KIR interface residue Glu106 and sweeps roughly parallel to the interface (Fig. 3B).

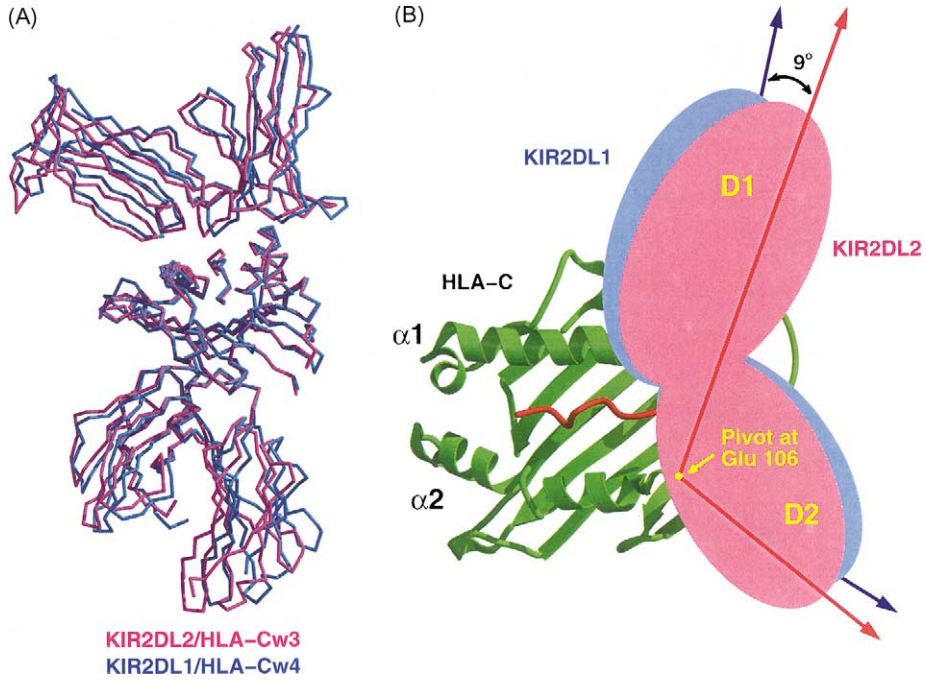

Fig. 3. Common binding mode of KIR2DL2 and KIR2DL1. (A) Cα trace representing the superposition of the KIR2DL2/HLA-Cw3 (magenta) and KIR2DL1/HLA-Cw4 (blue) complexes (based on the α1α2 HLA-C domains); (B) cartoon illustrating the 9° rotation between KIR2DL2 (magenta) and KIR2DL1 (cyan) when bound to HLA-C (green ribbon with a red peptide).

Relative to KIR2DL2, the D1 domain of KIR2DL1 rotates toward the C-terminus of the α1 helix and slightly away from the α1 helix as a whole. Likewise, the D1 domain of KIR2DL1 rotates toward the C-terminus of the α2 helix and slightly closer to the α2 helix. This orientation change results in relative translations of up to 2.6 Å for KIR interface α-carbons and conformational changes of several side chains. This also shifts the receptor interface boundary by two residues, but does not alter the HLA interface. Relative to KIR2DL2, the KIR2DL1 interface gains Arg68 and Tyr134 while it loses Glu21 and residue 70. The origin of this relative rotation is not clear and may result from a cumulative effect of residue differences at the interface, particularly those involved in specificity. Remarkably the KIR/HLA interface is able to accommodate these adjustments in receptor-ligand orientation, without disrupting the binding mode or reducing affinity. As a consequence, about half of the atom–atom contacts are conserved between the two complexes. However, many of the same side chain pairs interact in slightly different ways and the chemical nature

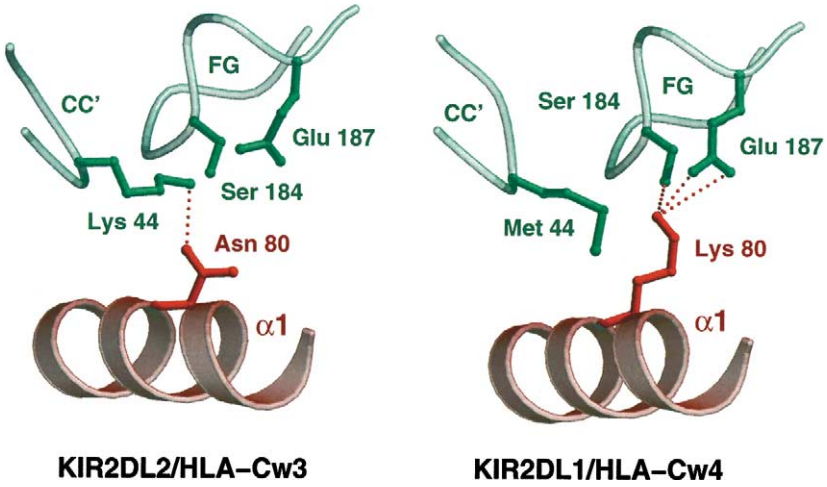

Fig. 4. Allotypic recognition by KIR2DL2 (left) and KIR2DL1 (right). Interactions between HLA-C residue 80 and KIR residues are highlighted in each panel. KIR is shown in green and HLA-C in brown. Hydrogen bonds and salt bridges are represented by dotted lines.

of most contacts are preserved between both complexes. For example, a salt bridge between Glu21 of KIR2DL2 and Arg69 of HLA-Cw3 is replaced by a hydrogen bond between Arg68 of KIR2DL1 and Gln72 of HLA-Cw4. Additionally, Van der Waals interactions between Met70 of KIR2DL2 and Arg69 and Gln72 of HLA-Cw3 are replaced by those between Tyr134 of KIR2DL1 and Ala149 of HLA-Cw4. These residues are all conserved except for KIR residues 68 and 70.

Comparisons between the KIR/HLA structures and structures of their free components reveal that only minor conformational changes take place upon complex formation. Although, the hinge angle between the D1 and D2 domains of KIR2DL1 increases by about 10° upon complex formation, the hinge angle of KIR2DL2 changes less than 2° upon binding HLA-Cw3. Furthermore, variations in the relative domain orientations observed in the crystal structures of ligand free KIR2DL1, 2 and 3 indicate limited intrinsic flexibility. Crystal structures, therefore, suggest that KIR and HLA associate essentially as rigid bodies. This is in contrast to TCR/MHC interaction that is characterized by conformational changes in the TCR binding loops upon binding.

4. Allotypic recognition of HLA molecules

Specificity is an integral characteristic of KIR/HLA interaction as various KIRs are restricted to particular groups of HLA alleles or allotypes. For example, KIR2DL1 and KIR2DS1 both interact with HLA-Cw2, 4, 5, 6, and 15, whereas KIR2DL2 and KIR2DL3 both recognize HLA-Cw1, 3, 7, and 8 allotypes. Earlier studies have implicated KIR2D residue 44 and the HLA-C heavy chain residue 80 as being critical in allotypic recognition (Winter and Long, 1997; Mandelboim et al., 1996). However, it was not until the crystal structure of the KIR2DL2/HLA-Cw3 complex was determined that the structural basis for allotype discrimination became clear. Interestingly, residue 80 is the only class I interface residue that varies among all HLA-C allotypes. It is an Asn in HLA-Cw1, 3, 7, and 8, but a Lys in HLA-Cw2, 4, 5, 6, and 15 allotypes, thus defining two receptor specificity groups. Of the 16 KIR interface residues observed in the KIR2DL2/HLA-Cw3 structure, all are conserved in the sequence of KIR2DL3 and two residues (44 and 70) differ in KIR2DL1. A third residue, amino acid 68, which is present only in the KIR2DL1/HLA-Cw4 interface also differs between KIR2DL2 and KIR2DL1. In the KIR2DL2/HLA-Cw3 complex, Lys44 of KIR makes a hydrogen bond to Asn80 of HLA-Cw3. KIR2DL1, which does not bind to HLA-Cw3, has a Met at position 44 instead and cannot make a hydrogen bond. In the KIR2DL1/HLA-Cw4 complex, Lys80 of HLA-C fits more deeply into KIR than Asn80, enabling it to make a salt bridge with Glu187, a hydrogen bond to Ser184 and hydrophobic interactions with Met44 and the aliphatic portion of Glu187 (Fig. 4). KIR2DL2 and KIR2DL3, each with a Lys at position 44 would have unfavorable electrostatic and steric interactions with HLA-Cw4 Lys80, thus destabilizing a KIR/HLA complex. In addition to KIR position 44, residues 68 and 70 also differ between KIR2DL1 and KIR2DL2. Arg68 in KIR2DL1 makes a hydrogen bond with Gln72 in HLA-Cw4. The Pro at position 68 in KIR2DL2 and KIR2DL3 precludes such contact. Met70 in KIR2DL2 makes minor hydrophobic contact with Arg69 of HLA-Cw3, but the smaller Thr at position 70 in KIR2DL1 makes no contact with HLA-Cw4 in its complex. Although, Arg68 helps stabilize the KIR2DL1/HLA-Cw4 complex and Met70 may modestly stabilize the KIR2DL2/HLA-Cw3 complex, it is clear that residue 44 is the key to KIR2DL/HLA-C specificity. A single Lys44 to Met mutation in KIR2DL2 and, conversely, a Met44 to Lys mutation in KIR2DL1 are each observed to be sufficient to switch allotype specificity (Winter and Long, 1997). Furthermore, residue 80 of HLA-C is the only interface residue which varies by the receptor allotype specificity. Accordingly, allotypic recognition of HLA-C molecules by KIR depends critically on specific interactions between KIR residue 44 and HLA heavy chain residue 80.

More than a dozen KIR genes from the human LRC have been identified, most of which are polymorphic (Trowsdale et al., 2001). Identifiable ligands include various class I HLA-A and -B allotypes and the non-classical class I molecule HLA-G in addition to HLA-C molecules (Dohring et al., 1996b; Pende et al., 1996; Wagtmann et al., 1995b; Rajagopalan and Long, 1999). Ten out of the 18 KIR interface residues are highly conserved among KIR2DL1, 2, 3, 4, and 5 and KIR2DS1, 2, 3, 4, 5, and 6 and KIR3DL1, 2, 3, and 7 and KIR3DS1 receptors (Fig. 5). These 10 residues all reside within the hinge region or the D2 domain that contacts the HLA α2 helix. In contrast, the less conserved KIR D1 domain appears responsible for HLA loci and allotype specificity through interaction with the α1 helix. This strongly suggests that the binding mode observed for KIR2DL1 and 2 is conserved among KIR molecules. Indeed, sequence comparisons of HLA-C alleles and HLA-B alleles reveals that all but three interface residues (α1 helix positions 69, 76, and 80) are conserved across HLA-B and C loci (Fig. 5). These three residues interact with all three KIR D1 loops in the two KIR/HLA complexes. Interestingly, KIR3DL1 recognizes multiple HLA-B alleles with the Bw4 serological epitope that spans residues 77–83 of the α1 helix in class I. Substitutions within this region significantly impact its recognition by KIR3DL1 (Gumperz et al., 1995, 1997). Similarly, HLA-B46, which contains the HLA-Cw1 sequence between residues 66–76 (within the α1 helix), is recognized by NK cells that express HLA-C specific receptors (Barber et al., 1996).

5. KIR/HLA binding displays a peptide preference

In addition to the class I MHC residues, the associated peptides are also crucial for KIR recognition (Wagtmann et al., 1995a; Zappacosta et al., 1997). TAP-deficient cells

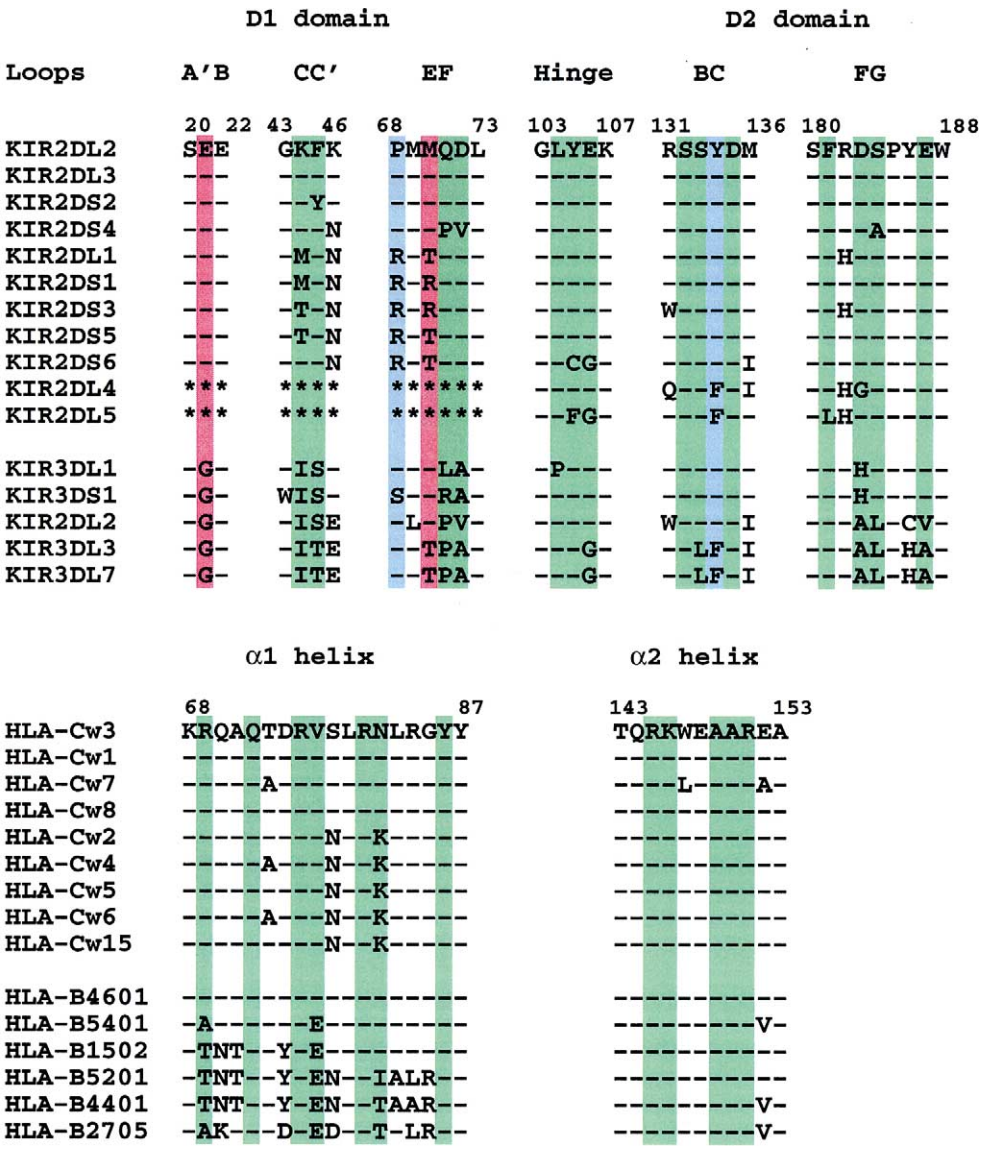

Fig. 5. Sequence alignments of the binding regions of KIR and HLA molecules. The six HLA binding loops of 16 KIR receptors are aligned to KIR2DL2 in the top panel. The bottom panel displays an alignment of HLA-C and Bw4 allotypes in reference to HLA-Cw3. Green shading indicates residues that interact in both the 2DL2/Cw3 and 2DL1/Cw4 complexes. Residues that interact only in the 2DL2/Cw3 complex are shaded pink and residues that interact only in the 2DL1/Cw4 complex are shaded blue. KIR2DL4 and KIR2DL5 do not have a D1 domain; instead they have a D0 domain, marked by asterisk (∗) in this figure.

cultured at 26 °C in the absence of class I binding peptides, express "empty" class I MHC molecules on their cell surface. These class I molecules are unable to protect the target cells from lysis, indicating a requirement for peptide beyond merely stabilizing the class I molecule. Moreover it is also evident that peptides vary considerably in their ability to enable a functional interaction between KIR and class I molecules. The KIR preference for peptides of certain sequence was first demonstrated in studies examining NK cell recognition of HLA-B27 expressing target cells. These studies found that certain residues at the P7 and P8 positions of the peptide failed to confer HLA-B27-dependent protection from NK cell-mediated lysis. In particular, charged residues such as, Asp or Arg at the P7 position and Glu or Lys at the P8 position rendered class I MHC more susceptible to lysis (Peruzzi et al., 1996a,b). Peptide preferences have also been observed in KIR2DL recognition of HLA-C molecules (Rajagopalan and Long, 1997; Zappacosta et al., 1997).

5.1. Peptide interaction with KIR

The crystal structure of KIR2DL2/HLA-Cw3 complex contains the nonamer peptide GAVDPLLAL (GAV) derived from the human importin-α1 subunit (Zappacosta et al., 1997) and the structure of the KIR2DL1/HLA-Cw4 complex contains the nine residue consensus peptide QYDDAVYKL

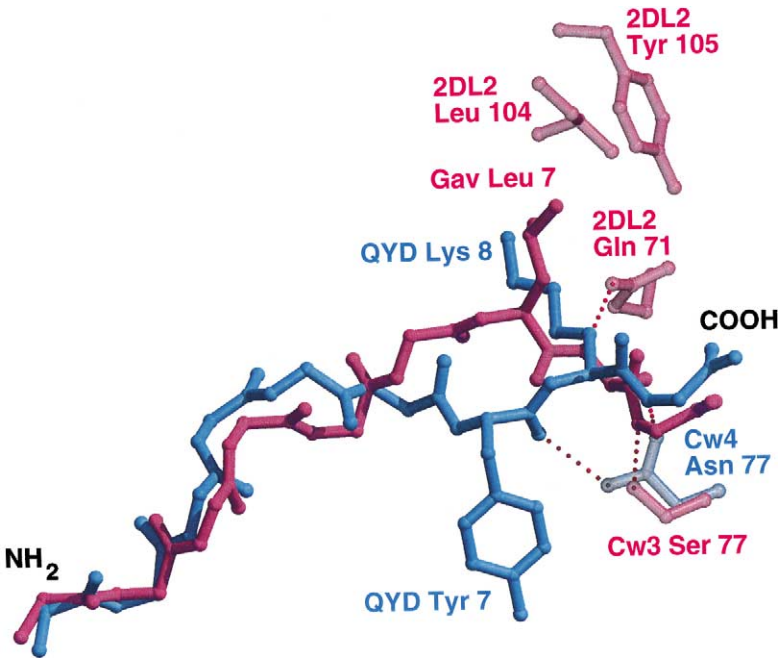

Fig. 6. Superposition of the GAV and QYD peptides. GAV (magenta) and QYD (blue) are represented by backbone stick models. Peptide side chains are shown for the P7 and P8 positions only. Selected KIR and HLA side chains that contact the peptide are also displayed. GAV interacting residues are light purple and QYD interacting residues are light blue. Hydrogen bonds are depicted by red dotted lines.

(QYD). Both bound peptides display the characteristic class I MHC peptide binding motif, in which the termini are secured by a conserved set of hydrogen bonds, the P2 and P9 residues are anchored in binding pockets and middle P4–P7 positions form an arch above the floor of the HLA-C cleft. In each complex, KIR is situated directly over peptide residues P7–P9. This results in direct contact between KIR2DL2 and the GAV peptide at positions P7 and P8. The Leu side chain at P7 of GAV projects upward away from HLA-Cw3 to form hydrophobic contact with KIR residues Leu104 and Tyr105 (Fig. 6). There is also sufficient space above P7 to accommodate larger amino acids such as Tyr observed in another protective peptide. Gln71 of KIR makes a hydrogen bond to the main chain nitrogen of the peptide Ala8 and hydrophobic contact with Cβ of the same Ala. This interaction brings Ala8 sufficiently close to KIR that it constrains the size of P8 side chain that would allow for KIR/HLA interaction.

5.2. Examination of P8 peptide preference

To further investigate the P8 peptide preference for KIR/HLA binding, substitutions were introduced at the P8 position of the GAV peptide (Boyington et al., 2000). Binding to the class I MHC specific antibody w6/32 demonstrated that these substitutions did not affect HLA-Cw3 stability. SPR binding measurements revealed that the affinity of KIR2DL2 for HLA-Cw3 decreases as the size of the peptide P8 side chain increases. In fact, P8 residues the size of Val or larger essentially abrogated KIR2DL2 binding (Table 1). Interestingly, HLA-Cw3 complexed with the nine residue TAMDVVYAL peptide, which differs from GAV except at the P4, P8 and P9 positions, the eight residue QAISPRTL peptide that differs at every position except P9, or the motif peptide AAADAAAAL, bind to the receptor with affinities not much lower than that of the wildtype GAV peptide. These results confirm that residues other than P7 and P8 do not contribute significantly to the receptor recognition. Furthermore, they suggest that Leu at P7 (GAV peptide) is only slightly more favored than an Ala, Tyr or Arg at this position.

5.3. Induced fit model for peptide interaction with KIR

The “upward projecting” conformation of the Leu side chain at the P7 position is a curious feature of the bound GAV peptide. A survey of the crystal structures of both human and murine class I MHC molecules complexed with nonamer peptides, indicates that the P7 position generally adopts a partially buried conformation with its side chain orientated roughly parallel to the floor of the MHC peptide binding groove or sometimes completely buried within the floor. The P7 peptide side chains in these structures range from 67 to 100% buried. In contrast, Leu7 of the GAV peptide in the KIR2DL2/HLA-Cw3 complex is only 38% buried by HLA-Cw3. This is particularly significant considering the hydrophobic nature of Leu and the complete burial of the P7 Tyr in the floor of the peptide binding cleft observed in the structure of KIR2DL1/HLA-Cw4. It is possible that KIR recognition induces a conformational change of the peptide such that the P7 position becomes more accessible

to the receptor. Indeed, as a result of the hydrogen bonding between Gln71 of KIR and the amide nitrogen of P8, the peptide backbone of GAV adopts a conformation slightly more puckered than other peptides in the vicinity of P7 position. A similar peptide conformational change can also be found in the Tax peptide when comparing the structure of HLA-A2/Tax with the structure of HLA-A2/Tax/A6 TCR complex (Ding et al., 1998).

Unlike the structure of KIR2DL2/HLA-Cw3, the peptide makes no direct contact with KIR in the KIR2DL1/HLA-Cw4 complex. Furthermore, the QYD peptide conformation between positions P4 and P8 diverges significantly from that of GAV (Fig. 6). The most noteworthy difference is observed at the P7 position. The QYD P7 main chain points down toward the floor of peptide binding groove, allowing complete burial of the Tyr7 side chain, whereas the GAV P7 main chain arches up towards the KIR receptor facilitating a direct interaction. The QYD peptide conformation observed in the crystal structure of receptor free HLA-Cw4/QYD is essentially identical to that of the complex, demonstrating that KIR2DL1 binding induces no conformational change of QYD. It is possible, however, that KIR binding does induce the unique conformation of the GAV peptide observed in the KIR2DL2/HLA-Cw3 complex. Although, the peptide interacting residues Gln71, Leu104 and Tyr105 are also present in KIR2DL1, the QYD Tyr7 may be too deeply buried to participate in a conformational change. Indeed, this Tyr is secured to HLA-Cw4 by four hydrogen bonds and 20 non-polar contacts of less than 4 Å. Despite making no contact with the peptide, KIR2DL1 imposes side chain restrictions on peptide residues P7 and P8. Experiments with synthetic peptides reveal that Asp or Glu at the P8 position or Glu at the P7 position significantly reduce KIR2DL1/HLA-Cw4 interaction (Rajagopalan and Long, 1997). In the crystal structure, the region of KIR2DL1 closest to these two residues is electronegative and non-polar in nature, providing an unfavorable environment for acidic side chains. Interestingly, the long Lys side chain at the P8 position of QYD in the complex structure actually extends toward the amino-terminus of the peptide all the way to the edge of the complex where its primary amine is solvent exposed.

5.4. Specific KIR/HLA pairs may constrain the peptide conformation

In summary, the interaction of both KIR2DL1 and 2 with their HLA-C ligands imposes constraints on the residues of the bound peptide P7 and P8 positions. However, in each case the detailed interactions are different. KIR2DL2 interacts with the GAV peptide such that there is only room for small side chains at the P8 position. KIR2DL1 on the other hand, by not directly interacting with QYD, provides space for larger amino acids, but with an electronegative environment not suitable for acidic residues. A key question remaining is the origin of the dramatically different peptide conformations observed in each complex that in turn impose limited peptide specificity for receptor binding. This is likely to depend on both the peptide/HLA interaction and peptide/KIR interactions. For example, Ser77 in HLA-Cw3 makes a critical hydrogen bond with the peptide main chain, stabilizing the conformation of the GAV peptide. In HLA-Cw4, residue 77 is an Asn that makes two conformationally significant hydrogen bonds to the main chain of the QYD peptide (Fig. 6). Moreover, residue 77 forms a dimorphic pair with residue 80 in HLA-C alleles (Fig. 5). Ser77–Asn80 is always present in the KIR2DL2 binding HLA-Cw1, 3, 5, and 7 allotypes, whereas the Asn77–Lys80 pair is present in KIR2DL1 binding HLA-Cw2, 4, 6, 8, and 15 allotypes. In addition, the different orientation of KIR2DL1 relative to KIR2DL2 in the HLA-C complex moves the Gln71 Cα 2.4 Å further from the peptide, making a hydrogen bond with the P8 main chain very improbable, arguing that the conformation of the bound peptide is integral to the specific KIR/HLA pair.

6. KIR and TCR recognition modes reflect functional differences

A distinguishing characteristic of KIR/HLA interaction is that the interface is more conserved than TCR/HLA interfaces. For example, only eight out of 16 HLA-A2 residues in contact with the A6 TCR are conserved among HLA-A alleles. This reflects the very different functions of KIRs and TCRs in the innate and adaptive immune system, respectively. KIR molecules recognize large groups of HLA alleles bound to diverse peptides, essentially detecting whether or not various HLA allotypes are being expressed on target cells. Clonotypic TCRs on the other hand recognize particular HLA/foreign-peptide complexes with high specificity in order to discern the presence of an intracellular pathogen. A vast array of TCR clones generated by gene rearrangement combined with the polymorphism of class I HLA molecules enables the adaptive immune system to oppose myriad pathogens. The antigen-driven clonal selection and expansion of T-cells, however, is time consuming. As part of the innate immune system, NK cells must respond rapidly to pathogens and do so through germline encoded receptors, such as KIR. Despite relatively low KIR diversity, however, effective immunosurveillance of class I HLA expression entails recognition of numerous HLA alleles, many more than the number of KIR receptor types available. KIR accomplishes this by recognizing conserved residues within polymorphic HLA molecules. This effectively enables HLA molecules to fulfill the recognition requirements for both the innate and adaptive immune systems.

It is worth noting that the footprint of KIR on HLA is distinct but overlapping with that of TCR (Fig. 7A). This becomes relevant on a subpopulation of T-cells, such as the NKT cells that express both KIR and TCR on their surface. The overlapping receptor binding site makes it impossible to

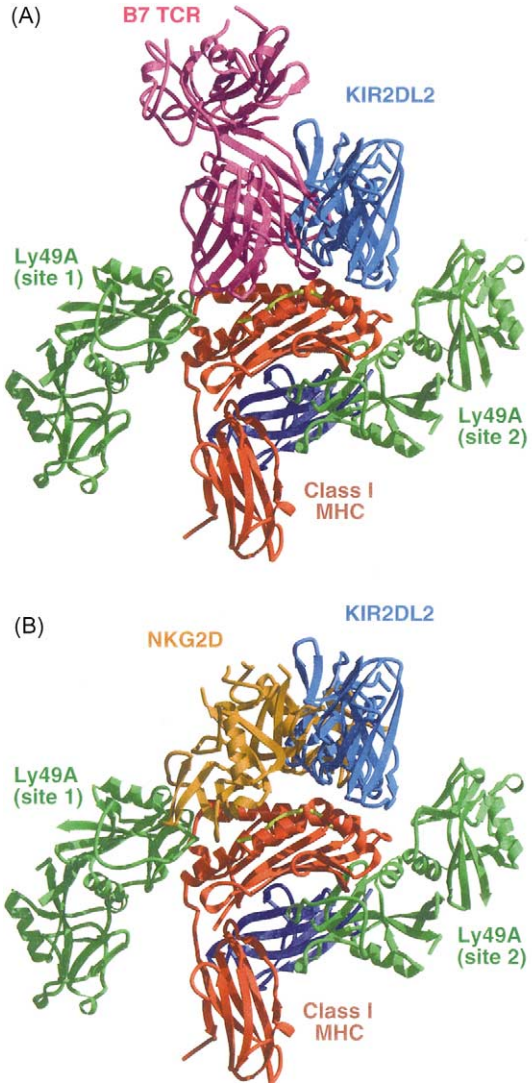

Fig. 7. Multiple modes for binding receptors to class I MHC molecules. (A) The Ly49A/H-2D^d complex (green, PDB code 1QO3) and the B7 TCR/HLA-A2 complex (magenta, PDB code 1BD2) are each superimposed with the KIR2DL2/HLA-Cw3 complex (red and blue, PDB code 1EFX) according to their MHC α1α2 domains. For clarity, only the HLA-Cw3 MHC is shown; (B) the same as in (A) except that the B7 TCR has been replaced by NKG2D (orange). NKG2D is oriented by the superposition of the NKG2D/ULBP3 complex (PDB code 1KCG) onto KIR2DL2/HLA-Cw3 through the ULBP3 molecule. For clarity, ULBP3 is not shown.

have both KIR and TCR bound simultaneously on one class I HLA molecule, thereby avoiding a potential conflict between the inhibitory signal by KIR and the activating signal by TCR.

7. KIR/HLA interaction displays similarities with NKG2D/ULBP interaction

Two crystal structures of the activating NK cell receptor NKG2D complexed with its MHC-like ligands, ULBP3 and MICA, respectively have been recently determined, revealing similarities with KIR/HLA binding (Li et al., 2001; Radaev et al., 2001). NKG2D is a homodimeric receptor of the C-type lectin-like receptor (CTLR) superfamily. ULBP and MICA are stress induced ligands each consisting of α1α2-like domains of MHC molecules with no bound peptide. MICA has an α3 domain, whereas ULBP does not. The relative orientation of the NKG2D/ligand complex is analogous to that observed in KIR/MHC and TCR/MHC complexes (Fig. 7B). The long axis of the NKG2D dimer binds diagonally across both α1 and α2 helices of the MHC-like ligands. Interestingly, one subunit of NKG2D binds the same area of the α1α2 domain as the KIR D1 domain, while the other NKG2D subunit binds the same α1α2 region as the Vα domain of TCRs. It is noteworthy that NKG2D binding is completely different from the binding of the murine inhibitory CTLR, Ly49A (Fig. 7B).

Unlike KIR/HLA interaction, however, the NKG2D/ligand complexes lack strong charge complementarity and the interfaces bury a significantly larger surface area than KIR/HLA complexes (approximately 2000 Å^2 versus 1500–1600 Å^2). Furthermore, in contrast to the reliance on sequence conservation seen in KIR/HLA recognition, the interface residues of ULBP1, 2, and 3 and MICA display low sequence identity, resulting in strikingly different interface interactions in the NKG2D/ULBP3 and NKG2D/MICA complexes. An induced fit model has been proposed to account for NKG2D ligand recognition (Radaev et al., 2001). Thus NKG2D and KIR each use drastically different ligand recognition mechanisms.

8. KIR/HLA recognition is fundamentally different from Ly49A/H-D^d recognition

The recent crystal structure of a complex between the murine NK cell inhibitory receptor Ly49A and H-2D^d has unveiled a very different receptor/MHC binding mode (Tormo et al., 1999). In contrast to KIR, whose footprint on HLA-C superficially resembles that of TCRs on the class I MHC molecules, Ly49A has two distinct binding sites on H-2D^d (Fig. 7). The first binding site is located at the N-terminal region of the class I MHC α1 helix, whereas the second binding site is located below the H-2D^d peptide binding groove in a region that interfaces with α2, α3 and β2m of H-2D^d. Site 2 also partially overlaps with the binding site for CD8. No direct peptide contacts between Ly49A and H-2D^d are observed in either of the interfaces. Site 1 was favored initially since the orientation of the receptor at this site is consistent with the positioning of the effector NK cells even though site 2 buries a more extensive receptor-MHC interface. Mutations in the regions of site 1 and 2 were produced recently to test the validity of each site (Chung et al., 2000; Matsumoto et al., 2001). The results from Chung et al. (2000) favored site 1, whereas

the opposite conclusion was reached by Matsumoto et al. (2001). Further mutational and structural studies are needed to definitively resolve whether site 1 or 2 or both sites are important for the receptor recognition. In any case, the molecular details underlying class I recognition by murine Ly49 receptors appear to be very different from those governing KIR recognition despite their functional similarities. It also remains to be seen whether the MHC binding mode of Ly49A is conserved among other Ly49 molecules, such as Ly49C that unlike Ly49A, recognizes H2-K^b in a manner sensitive to the peptide sequence. Similarly the question arises as to whether other class I recognizing CTLRs, such as the CD94/NKG2 receptors interact with class I in a manner that resembles that of Ly49A receptor with class I. In particular, all members of the CTLR superfamily of receptors appear to function as dimers and the known structures of this superfamily share a conserved dimerization mode originally described in the structure of CD94 (Boyington et al., 1999; Tormo et al., 1999; Natarajan et al., 2000).

9. A Model for KIR/HLA clustering during immune synapse formation

Ligand induced receptor oligomerization is presumed to be a common mechanism for initiating receptor-mediated signaling. Some of the best understood examples to date are the growth hormone mediated dimerization of human growth hormone receptor and the erythropoietin(EPO) -induced conformational change in the EPO receptor (de Vos et al., 1992; Livnah et al., 1996). Since KIR receptors bear a structural resemblance to hematopoietic receptors, it has been proposed that KIR may form dimers upon binding to HLA in a manner similar to the growth hormone receptor. However, the crystal structure of KIR/HLA complex and the previous solution studies demonstrated a 1:1 stoichiometry for KIR/HLA interaction (Boyington et al., 2000; Fan et al., 1996). Observations of immunological synapses at the interface of T-cells and APC have revealed a central cluster of TCR/MHC complexes and a peripheral ring of adhesion molecules (Monks et al., 1998; Grakoui

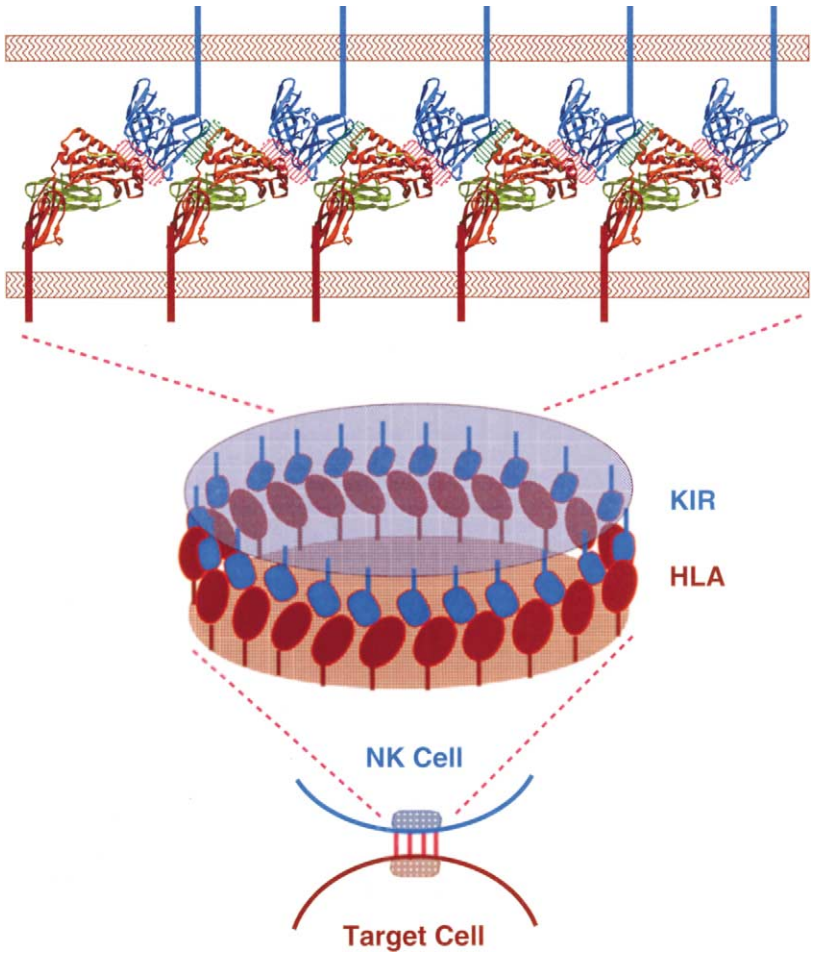

Fig. 8. A model for HLA induced KIR clustering. The top panel shows the oligomeric aggregate within the crystal lattice. HLA-Cw3 and KIR are brown and blue, respectively. The red and green hashes highlight the functional and aggregate interfaces, respectively. Horizontal brown hash above and below the aggregate depict the NK cell and target cell membranes, respectively. The bottom two panels show cartoons depicting the KIR/HLA aggregate on the periphery of the NK cell immune synapse.

et al., 1999). In contrast, the observed NK cell immune synapse is formed with a central LFA-1/ICAM-1 cluster and a peripheral KIR/HLA cluster in a shape of a donut (Davis et al., 1999). The question of how KIR/MHC complex forms this ordered donut-shaped oligomeric aggregate remains unclear. One possible form of such an oligomer was reported in the crystal structure of KIR2DL2 (Snyder et al., 1999). In that model, the receptors form a regular array through an interaction between the D1 domain of a receptor and the D2 domain of the preceding receptor. A second oligomerization form was observed in the crystal structure of the KIR2DL2/HLA-Cw3 complex (Boyington et al., 2000). Each KIR molecule within the crystal lattice makes an additional contact, apart from the functional binding interface, with a symmetry related HLA-Cw3 molecule in a peptide independent manner. This KIR/HLA contact surface is formed between the B and E β-strands of the KIR D2 domain and the C-terminal end of the α2 helix of HLA-Cw3 (Fig. 8). The interface buries 530 $Å^2$ of surface area and is characterized by mostly Van der Waals interactions. Interestingly, this KIR/HLA contact bridges adjacent complexes to form an oligomeric KIR/HLA aggregate (Fig. 8). In this form of oligomer, the KIR/HLA complexes are all in the same orientation and the receptor and ligand stoichiometry is maintained at 1:1. Furthermore, the putative glycosylation sites on both KIR2DL2 and HLA-Cw3 are located away from the oligomerization interface. It is possible that this form of receptor-ligand oligomerization resembles the receptor clustering on the surface of natural killer cells. Additional studies are clearly needed to address the biological relevance of this oligomer, particularly its implications for receptor signaling.

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